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Soil predator loss alters aboveground stoichiometry in a native but not in a related range-expanding plant when exposed to periodic heat waves

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ABSTRACT

Increasing frequency and magnitude of climatic extremes, such as heat waves are expected to enhance abiotic stresses on ecological communities. It has been proposed that ecological communities in disturbed habitats may be most sensitive to climatic extremes, as disturbance may reduce density and diversity of higher trophic level organisms like predators. However, there is little experimental evidence that climatic extremes indeed have stronger impact on functioning of such trophically downgraded ecosystems. Here, we experimentally examine how removal of predators from soil communities affects plant performance under periodic heat waves. We used a native plant species, and a congeneric native that is currently expanding its range because of climate change. We used soil nematode communities as the model system, as these are most abundant soil animals and their communities are trophically diverse. Predatory nematodes were manually removed from intact soil nematode communities (mainly the adults as some juveniles are impossible to manually remove) to create a trophically downgraded soil. Intact nematode communities and communities with reduced predatory nematodes were added separately to soils that were planted with either the native *Centaurea jacea* or the range-expanding congener *Centaurea stoebe*. Half the experimental units were exposed periodically to experimental heat waves of 10 °C above the control temperature. Our results show that the C: N and C: N: P ratio of plant shoots in predator-reduced soils became lower when exposed to periodic heat waves, however, only in the native plant *C. jacea*. The decrease in C: N ratio corresponded with increase of an herbivorous nematode in trophically intact soils of *C. jacea* independent of warming, whereas this relationships disappeared in warmed and predator-reduced soils. Our results accordingly highlight that periodic heat waves may affect stoichiometry of certain plant species by altering trophic interactions in predator-reduced soils.

1. Introduction

Anthropogenic climate warming continues to alter biodiversity and ecosystem functioning in many ecosystems (Pecl et al., 2017; Thakur et al., 2017; Yvon-Durocher et al., 2015). The effects of climate warming are particularly negative in disturbed landscapes where communities are often homogenous and low-diverse (Thakur et al., 2017; Tuff et al., 2016). A common biotic response in such landscapes is lower density and/or diversity of predators given their higher sensitivity to disturbances (Estes et al., 2011; Odum, 1985; Voigt et al., 2003). Such reduced density and/or diversity of predatory species in ecosystems is commonly referred to as trophic downgrading (Estes et al., 2011). Trophic downgrading can alter the community structure by changes in the dominance within prey communities via the loss of keystone predators (Harley, 2011; Valiente-Banuet et al., 2015), which could subsequently alter primary production of ecosystems (Estes et al., 2011; Zarnetske et al., 2012). Climate warming can further exacerbate trophic downgrading effects on ecosystems by increasing the vulnerability of already dwindling predator species in disturbed landscapes. However, the current understanding of the effects of trophic downgrading on ecosystems in a warmer world is mainly limited to studies of large predators living aboveground and in aquatic ecosystems (Estes et al., 2011; McCauley et al., 2015; Ripple et al., 2014).

Belowground (or soil) sub-ecosystems harbour a wide range of

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invertebrate predators (Bardgett and Van Der Putten, 2014; Thakur and Geisen, 2019). These invertebrate predators contribute to several ecosystem functions in soils, such as the cycling of elements and plant productivity (Orgiazzi et al., 2016). Most of the soil invertebrate predators either feed on microbial-feeding or on plant root-feeding organisms (Wardle et al., 1998). Plants are often indirectly affected by soil invertebrate predators (Moore et al., 2003). For example, soil predators feeding on plant root-feeding soil organisms can directly enhance plant performance by reducing the herbivore pressure on plants (Barber et al., 2015). Some soil predators feeding on microbial-feeding soil organisms can increase microbial biomass via trophic cascades, which could influence nutrient turnover rates in soils (Cragg and Bardgett, 2001; Thakur et al., 2015). If increase in microbial biomass constrains the release of nutrient turnover, this could reduce plant growth *via* the reduced availability of soil inorganic nutrients.

Soil invertebrate predators also show greater vulnerability to disturbances, such as to agricultural intensification and enhanced soil tillage than their prey species (Newbold et al., 2020; Postma-Blaauw et al., 2010; Tsiafouli et al., 2015). Recent studies have further highlighted that predacious soil invertebrates have greater vulnerability to climate warming in homogenous and disturbed landscapes where their density and diversity are already lower (Siebert et al., 2019; Thakur et al., 2017). We expect that predator losses in soils could amplify the climate warming effects on plants. For example, warming enhances nutrient turnover rates by increasing the feeding activity of microbial-feeding organisms (Pritchard, 2011). Loss of soil predators is likely to enhance nutrient turnover rates (Moore et al., 2003; Thakur and Geisen, 2019; Wardle, 2002). Taken together, warming and predator loss could make nutrients more accessible to plants with implications for their growth. In contrast, in the absence of soil invertebrate predators that feed on plant-root feeding organisms, plants may suffer more from an enhanced root herbivory, which also increase with warming (Tsunoda et al., 2018). Shifts in root herbivory can further affect plant's nutrient uptake and thus plant production and stoichiometry (Erb and Lu, 2013; Gebremikael et al., 2016; Johnson et al., 2016).

Among soil invertebrate predators, free-living soil nematode predators are one of the most abundant and diverse predatory groups (Quist et al., 2019; Thakur and Geisen, 2019). Importantly, these predators feed both on microbial-feeding and plant root-feeding soil nematodes (Yeates et al., 1993). Loss of these predators can potentially influence plant's performance via affecting changes in microbial-feeding nematodes or root-feeding nematodes. That is, loss of predatory nematodes can have an impact on soil nutrient availability as well as on root herbivory-both associated with an overall performance of plants. Recent research has indicated that trophic downgrading effects in soils on plant performance may depend on plant identity (Thakur et al., 2015; Wilschut, Geisen, ten Hooven and van der Putten, 2016). For example, loss of predator density only affected nutrient availability in the soil of a leguminous plant, but not so in an herbaceous or a grass species (Thakur et al., 2015). Another study recently reported differences in the strength of top-down effects on microbial- and root-feeding nematodes between range-expanding and native plants (Wilschut et al., 2016). These differences in the ability of nematode predators to control the microbial- and root-feeding nematodes could thus depend on the differences in plant's ability to accumulate organic resources in the soil, which controls the magnitude of top-down effects by predators on their prey (Oksanen et al., 1981; Thakur and Eisenhauer, 2015).

In this study, we aim to investigate how the combined loss of predatory nematodes and climate warming affects the performance of a range-expanding plant species and its congener native. Range expansion is a widely observed response of plants to on-going climate warming (Chen et al., 2011; Pecl et al., 2017). The success or failure of range-expanding plant species is expected to depend on both biotic and abiotic environments they are confronted with in the new region (Svenning et al., 2014; Van der Putten, 2012). We use predator loss as a characteristic of disturbed habitats, and periodic heat wave events as climate extreme, as such extreme weather events are becoming more common worldwide (IPCC et al., 2018) and are considered a serious threat to biodiversity and ecosystem functioning (Harris et al., 2018; Soroye et al., 2020). Newly arriving range-expanding plants are likely to be more naïve towards biotic interactions in the new range compared to native plants (Heger et al., 2019; Verhoeven, Biere, Harvey and van der Putten, 2009). Therefore, we expected that the interactive effects between the loss of predatory nematodes and periodic heat waves will be more pronounced for the performance of the native plant species than of the range-expanding plant species. Specifically, we hypothesize that the loss of predatory nematodes would enhance plant growth and nutrient uptake if microbial-feeding nematodes increase in the absence of predators. The impact of predatory nematode loss, however, could be negative for plants if plant-feeding nematodes increase in the absence of predators. We measured plant performance in terms of plant biomass and plant stoichiometry, as both are likely to be influenced by alterations in soil biotic interactions due to predator loss and climate warming.

2. Material and methods

2.1. Plants

We used two congeneric plants in this experiment: *Centaurea jacea* L. s.l. and *Centaurea stoebe* L. *Centaurea jacea* is a common native plant species in the Netherlands, whereas *C. stoebe* is a range-expander that is native in Central-South-Eastern Europe (Wilschut et al., 2016; Wilschut et al., 2019). Some populations of *C. stoebe* have recently established in the Netherlands in the areas of riparian vegetation close to where *C. jacea* also occurs. These two plant species were therefore selected to represent a realistic scenario of how trophic downgrading can influence a range-expanding and the related native plants, which are already co-occurring in nature. We thus collected the seeds of both plant species in a location of the Netherlands (see below) where the populations of both plants co-occur.

2.2. Soils

In August 2018, we collected soil from a riparian grassland at Vlietberg, the Netherlands (51° 86′ N, 5° 89' E) where both *C. jacea* and *C. stoebe* co-occur. Soil was carefully sieved to remove stones and roots and was subsequently sterilized using gamma-irradiation (25 kGy; Synvergy Health, Ede, The Netherlands) to eliminate all soil biota. The sterilized soil was then used as the substrate for the experiment.

2.3. Microbial and nematode inoculum

To prepare soil microbial and free-living soil nematode inoculum, we collected additional 25 kg of soil at the end of the main growing season (September 2018) from the same location of soil sampling. Soils were stored at 4 °C immediately after the collection from the field. A microbial inoculum was obtained by sequentially sieving 5 kg of this soil (as a water solution) through a series of decreasing mesh sizes. The sieving by the smallest mesh size of 20 μm was repeated five times to ensure the exclusion of all nematodes. We confirmed this by inspecting the inoculum using a dissecting microscope.

From the remaining 20 kg soil, in batches of 500 g (40 suspensions of nematodes in total), free-living soil nematodes were extracted by using an elutriator and cotton wool filters (Oostenbrink, 1960). This method of nematode extraction includes a step where nematodes actively move through a cotton wool filter into water, excluding dead or non-active nematodes. Two kinds of nematode inoculum from the nematode suspension were prepared: one with intact nematode communities and another with significantly decreasing the predatory nematodes. The major groups of predatory nematodes that were removed were from the order Mononchida and Dorylaimida, which are generally larger in size

than other nematodes (Supplementary figure 1; Mulder et al., 2011; Yeates et al., 1993) and are also more vulnerable to disturbances (Bongers, 1999; Holterman et al., 2008). Predatory behaviour in Mononchida and Dorylaimida is prominent in their adult stages, whereas their juveniles feed on smaller prey items such as soil microorganisms. The adult stages are also relatively easier to recognise under lower magnification (e.g. 40x) and therefore easier to remove from the suspension. Mononchida can be recognized by their relatively big barrel- or funnel-shaped mouth cavity and well-developed muscles surrounding the oesophagus, whereas Dorylaimida individuals have a well-developed lip region with a spear or a tooth (a protractible piercing device). Juvenile predators on the other hand are extremely hard to detect by microscopic inspection, and therefore were not removed from the suspension.

To have a rough initial separation of body sizes, nematode suspensions were collected after 24 h, 48 h, 72 h and 96 h on the cotton-wool filter. The proportion of large-body sized nematodes is assumed to become relatively higher than small-body sized nematodes with suspension time (De Goede and Verschoor, 2000). That is, in the 96 h and 72 h suspensions, the total numbers of nematodes are expected to be much lower, and the relative number of predators (which are often bigger in size) are expected to be higher than the 24 h and 48 h suspensions (De Goede and Verschoor, 2000). The long-duration suspensions therefore facilitated the detection of predatory nematodes, and thereby helped in their manual removal. Please note though that we manually removed adult predators from suspensions collected at all time points. Each of the 40 suspensions was split into two parts: the first part was for the intact nematode community; the second part was for the predator-reduced nematode community. A home-made nematode fishing device (a pig hair attached to a small wooden stick) was used to pick and remove predatory individuals from the suspension under a dissecting microscope at 20× magnification. In total ~1500 predatory nematode individuals were removed from a total of ~250,000 nematode individuals (~0.6% of the total nematode community). Nematode suspensions collected at various time points from the first part were mixed, and used as the intact nematode community, which included all feeding types (predators, omnivores, plant-feeding, bacterial-feeding and fungal-feeding nematodes). Nematode suspensions from various suspension time points from the second part were mixed together after adult predators were manually removed from each suspension, and used as the predator-reduced nematode community containing a decreased density and/or diversity of predators. We counted nematode individuals in the two inoculum types at 20× magnification and found ~5000 individuals per inoculum. Further, to check the efficiency of predator removal, two subsamples were taken quantitative PCR (qPCR) analysis from each nematode inoculum type. The overall nematode diversity prior to inoculation was assessed by using 48 qPCR primer sets to detect and quantify nematodes at species, genus, or family level (Supplementary Table 1).

2.4. Experimental set-up

The experiment consisted of three kinds of treatments: only microbial inoculum (no nematodes), nematode communities with reduced number of predatory nematodes, and intact nematode communities. These three inoculums were added to 1 L pot filled with 700 g of sterilized soil. Before the addition of three inoculums, we planted half the pots with *C. jacea* and another half with *C. stoebe*. Each pot received an individual seedling (2–3 cm high) of either of the plant species. Nematode inoculums contained ~5000 individuals of nematodes, corresponding to ~700 individuals per 100 g of soil, which is comparable with the nematode density under natural conditions (Quist et al., 2019).

The two plant monocultures in combination with three nematode treatments were grown for eight weeks and were exposed to two kinds of warming scenarios (Supplementary figure 2): 1) Ambient temperature of 20 °C at day (16 h) and 17 °C at night (8 h) for the entire eight weeks, and 2) Periodic heat waves with +10 °C the ambient temperatures (i.e. 30 °C at day (16 h) and 27 °C at night (8 h)) in weeks 3, 5 and 7. The periodic heat waves represent an extreme climatic event scenario in several regions of the Western Europe including the Netherlands (IPCC et al., 2018). We added the same volume of water (~50 ml per pot) every second or third day to keep similar levels of soil moisture across the treatments. Each climate scenarios were run in two climate chambers separately with each of them receiving all plant and nematode treatment combinations. We monitored both temperature and relative humidity regularly to check the efficiency of climate chambers (Supplementary Table 2). During the weeks of heat waves, the relative humidity was on average + 20% higher than in the ambient climate chambers (Supplementary Table 2). Each treatment combination was replicated eight times totalling in 96 microcosms (2 plant monocultures × 2 temperature scenarios × 3 nematode treatments × 8 replicates).

2.5. Experiment harvest

2.5.1. Plant biomass

At the end of the eighth week of the experiment, plants were large enough to cause pot limitation in affecting their growth whereas their sizes were also limiting the space in climate chambers. Thus, we harvested plants at the end of the eighth week by clipping the plant shoots at the soil surface. Prior to biomass measurements plant roots were thoroughly washed to remove all soil particles (portion of which were collected for other measurements, see below) from each pot. Both shoots and roots were dried at 40 °C for at least 72 h before their dry weight was measured.

2.5.2. Nematode communities

Nematodes were extracted from 200 g soil containing the plant root using the elutriator-cotton wool filter method over three days (Oostenbrink, 1960). The nematode suspensions were then concentrated, and DNA was extracted by a lysisbuffer (Vervoort et al., 2012), and DNA extracts were purified using a glass fibre column-based procedure (Ivanova et al., 2006). Quantitative PCR was performed in a 20 μl final volume, containing 3 μl of 10x diluted purified DNA extract, 1 μl of each of the nematode taxon-specific PCR primers (200 μg/μl), 5 μl Milli-Q water and 10 μl IQ SYBR Green Fluorescein Mix (Bio-Rad). The following qPCR program was used on a Bio-rad CFX, t: 95 °C for 3 min to activate the DNA polymerase, followed by 50 repeats of the three qPCR steps: denaturation (95 °C for 30 s), annealing (63 °C for 60 s) and extension (72 °C for 30 s), followed by a melting curve program (from 72 to 95 °C with steps of 0.2 °C). We assessed the overall nematode diversity before inoculation and at the end of the eighth week of the experiment by using 48 nematode-taxon specific primer sets. The 48 primer sets were selected based on prior knowledge about the diversity of nematode communities in a similar vegetation in the same area (Quist et al., 2019). We used 48 primer sets to perform a biodiversity assessment including 46 primer sets to detect and quantify 46 different nematode taxa. One primer set was used to qPCR-count the total densities and other primer set was used as internal control. Before inoculation, the templates for the biodiversity assessment with 48 primer sets were subsampled from the two inoculum types. At the end of the experiment, a mixture of DNA from each of the 48 samples per inoculum type was made (this mixture was obtained by mixing a small portion (3 μl) of each of the 48 purified DNA samples together). A subset of the qPCRs resulted in a positive signal; at the start of the experiment, 18 of the 46 taxa were detected in the inoculum, whereas 11 taxa from 46 taxa were detected at the end of the experiment (Supplementary Table 1). The 11 primer sets were selected to detect and quantify the corresponding 11 taxa in each of the 96 soil samples individually.

Quantitative PCR reactions were performed, and the Cycle-threshold (C_t) values were converted to nematode densities by making use of the known linear relationships between C_t values and the ^{10}log (number of target nematodes). The known linear relationships were obtained by

performing qPCR reactions on DNA from known densities of nematodes from a given taxon (Holterman et al., 2008; Vervoort et al., 2012; Quist et al. 2017; Quist et al., 2019). The maxima of the negative, first mathematical derivative of the melting curves were checked to confirm the correct nature of the amplicons (Vervoort et al., 2012). Based on the outcome of the nematode diversity assessments, 12 primer sets were selected to detect and quantify 11 nematode taxa and the overall nematode densities in the 96 samples by qPCR (Supplementary Table 1). At the end of the experiment, a number of samples from pots that received an inoculum without nematodes were selected randomly and were microscopically inspected confirming the absence of nematodes in these samples.

2.6. Analysis of microbial biomass

From each pot, 1 g of mixed soil was used for the extraction of microbial DNA (on the principle of the MoBio PowerSoil DNA isolation kit). The 1 g soil was added to 3 mL bead solution and 0.24 mL of a lysis buffer containing Sodium dodecyl sulfate (SDS). Five iron spheres (diameter: 3 mm) and 1 g of silicon carbide powder (grit 46) were added to enhance the extraction efficiency. Tubes were shaken for 20 min at 2850 rpm in a bead beater. After centrifugation, humic acids were removed using 0.8 mL of an ammonium aluminium sulfate dodecahydrate solution. DNA extracts were purified using a glass fibre column-based procedure (Ivanova et al., 2006). Total bacterial DNA and fungal DNA were quantified by qPCR and Ct values were converted to biomass (ng DNA) as described by (Harkes et al., 2017).

2.7. Analysis of abiotic data and stoichiometry

At the end of the experiment, the dried plant shoots and the dry soil from each experimental pot were analysed for C, N and P content. Plant shoot materials were ground into powder and about ~3 mg (using a microbalance: Sartorius microbalance ME5) of this powder was carefully placed in tin capsules from each samples. The C and N concentrations from these tin capsules were estimated using an elemental analyser (Flash EA 1112, Thermo Scientific) following the Micro-Dumas combustion method. For the P content estimation in shoot materials, we used methods explained in Murphy and Riley (1962) to estimate the phosphate concentration. In short, the organic matrix in shoot material (3–4 mg) is destroyed by ignition of the sample in a muffle furnace. Then the total amount of phosphorus is released and converted into orthophosphate by digestion in an autoclave with a 2.5% potassium persulfate solution. The phosphorus content in the shoot material expressed in percentage of P was calculated as P (%) = (C * K)/(10 * Y), where C is phosphate concentration (mg/L), K is the volume of potassium persulfate solution (mL) and Y is the weight of shoot material used for digestion (mg).

2.8. Data analysis

We used two-way analysis of variance (ANOVA) to test the interactive effects of nematode treatments and periodic heat waves on the performance of two plants. Models to test plant performance were run separately for two plants using Gaussian error terms. Plant performance was assessed in terms of their dry biomass (shoot and root biomass) and shoot stoichiometry (C: N ratio, C: P ratio, N: P ratio and C: N: P ratio). Both total bacterial DNA and fungal DNA were also analysed using two-way ANOVA with the Gaussian error terms. The variation in nematode abundance of various feeding groups at the end of experiment was also analysed using two-way ANOVA models (with nematode treatments and heat waves as the fixed effect) with negative binomial error terms to account for the over-dispersion in the count data. We estimated the effect size of each treatment effect on a given response using partial omega-squared (ω^2-partial) (Olejnik and Algina, 2003). We further performed post-hoc Tukey tests on response variables for comparing the mean differences among the treatments. We also used Pearson correlation tests to examine potential associations among plant and nematode response variables from the final harvest. These tests were informed by the ANOVA models.

All statistical analyses were performed in the R statistical software (R Core Team, 2018). Linearity assumption for all models was tested using the test of homogeneity in variance using the DHARMa package (Hartig, 2017). The overdispersion in the count data was also tested using the DHARMa package (Hartig, 2017). The F-values were obtained using the Type II sum of squares test from the car package (Fox and Weisberg, 2011). The Tukey post-hoc test was performed using the multcomp package (Hothorn et al., 2008).

3. Results

3.1. Nematode inoculum and responses

From the biodiversity assessments in the two nematode inoculum types, we found that manual removing of adult nematodes had variable reductions among the major groups of predatory and omnivore nematodes. For instance, we found ~87% less *Dorylaimida D2*, ~35% less *Mononchida M3*, and ~27% less *Dorylaimida PP1* in predator reduced inocula than in predator intact inocula (Supplementary Table 1). Predator removal, however, did not reduce Dorylaimida D1 and Dorylaimida D3 when compared with intact inoculum (Supplementary Table 1). Please note that qPCR detects all developmental stages (both juveniles and adults) in the community, and it should therefore be noted that the results in Supplementary Table 1 might show an underestimation of the reduction of nematodes with predatory behaviour in our inoculums.

From the biodiversity assessments of nematode communities after the experiment, we found that *Coslenchus* – a genus of root hair feeders (Yeates et al., 1993) – was the most abundant plant feeding nematode in our experiment (Supplementary Table 1). The densities of plant feeding nematode (*Coslenchus*) were higher in the presence of predatory nematodes but only so in *C. jacea* plants at the end of the experiment (Table 1, Fig. 1). In contrast, we found no effect of periodic heat waves on these plant-feeding nematodes in either of the plant species' soil (Table 1, Fig. 1). Densities of both bacterial- and fungal-feeding nematodes at the end of the experiment did not significantly differ among any combination of treatments (Table 1). Although bacterial-feeding nematodes, such as *Mesorhabditis* and *Cephalobidae* had dramatically increased during the experimental period (Supplementary Table 1). Many predatory and omnivorous nematodes decreased on average during the experimental period across treatments (Supplementary Table 1). One of the omnivores *Dorylaimida D2* also showed a strong increase over the experimental period but mainly so in predator reduced communities compared to predator intact communities (306.78% increase vs. 3.36% increase, Supplementary Table 1). The overall density of predatory and omnivore nematodes was significantly affected by a negative effect of periodic heat waves but only in *C. stoebe* plant (Table 1).

3.2. Plant and microbial responses

Among plant biomass measurements, we only found root biomass of *C. jacea* to significantly respond to interactive effects of periodic heat waves and predator removal in the soil (Table 1). More specifically, root biomass of *C. jacea* was significantly lower when exposed to periodic heat waves but only so in the treatment where predatory nematodes were reduced (Supplementary figure 3). The shoot: root ratio of *C. jacea* was significantly higher in periodic heat wave treatments when predators were reduced (Supplementary figure 4). Other plant biomass measurements of *C. jacea* and *C. stoebe* showed no significant response to nematode community or temperature regime (Table 1).

In contrast, we found plant stoichiometry (namely the shoot stoichiometry) responding more strongly to periodic heat waves and reduced predators in the soil, but this effect was more pronounced in the

Table 1
Results of two-way ANOVA on plant performance (biomass and shoot stoichiometry), soil microorganisms and nematode densities affected by experimental manipulation of nematode community and periodic heat waves. The bold F- and p-values indicate statistical significance ($p < 0.05$), whereas we show the effect size using partial-ω^2 for treatments and their interactions effects. For nematode density responses, nematode community with two levels were used (predator reduced and predator intact), whereas for the rest, three levels of nematode community were used (no nematodes, predator reduced and predator intact). The sign in brackets next to effect sizes indicates the direction of the treatment effect. df stands for the degrees of freedom.

	Centaurea jacea									*Centaurea stoebe*								
	Nematode community (Nem)			Heat wave (H)			Nem×H			Nematode community (Nem)			Heat wave (H)			Nem×H		
	F-value$_{df}$	p-values	effect size (ω^2)	F-value$_{df}$	p-values	effect size (ω^2)	F-value$_{df}$	p-values	effect size (ω^2)	F-value$_{df}$	p-values	effect size (ω^2)	F-value$_{df}$	p-values	effect size (ω^2)	F-value$_{df}$	p-values	effect size (ω^2)
Nematode density																		
Bacterial-feeding nematodes	$0.22_{1,27}$	0.63	0.02	$1.84_{1,27}$	0.18	0.01	$0.04_{1,27}$	0.82	0.03	$0.11_{1,28}$	0.73	0.02	$3.84_{1,28}$	0.05	0.06	$0.14_{1,28}$	0.70	0.02
Fungal-feeding nematodes	$2.18_{1,27}$	0.15	0.03	$1.09_{1,27}$	0.30	0.01	$0.08_{1,27}$	0.77	0.01	$0.08_{1,28}$	0.76	0.02	$2.93_{1,28}$	0.09	0.05	$0.55_{1,28}$	0.46	0.01
Plant-feeding nematodes	**$9.69_{1,27}$**	**<0.01**	**0.12 (+)**	$0.45_{1,27}$	0.50	0.03	$0.47_{1,27}$	0.49	0.03	$2.44_{1,28}$	0.12	0.01	$3.98_{1,28}$	0.05	0.06	$0.87_{1,28}$	0.35	0.03
Predator/ omnivore nematodes	$1.99_{1,27}$	0.16	0.01	$<0.01_{1,27}$	0.94	0.03	$0.36_{1,27}$	0.55	0.02	$<0.01_{1,28}$	0.99	0.03	**$12.23_{1,28}$**	**<0.01**	**0.25 (-)**	$0.47_{1,28}$	0.49	0.01
Plant biomass																		
Shoot biomass	$0.70_{2,42}$	0.49	0.01	$<0.01_{1,42}$	0.98	0.02	$0.37_{2,42}$	0.69	0.02	$2.69_{2,42}$	0.07	0.06	$0.14_{1,42}$	0.70	0.01	$0.61_{2,42}$	0.54	0.01
Root biomass	$1.62_{2,42}$	0.2	0.02	$3.03_{1,42}$	0.08	0.04	**$3.33_{2,42}$**	**0.04**	**0.08 (-)**	$0.52_{2,42}$	0.59	0.02	$0.72_{1,42}$	0.39	0.01	$2.82_{2,42}$	0.07	0.07
Total biomass	$1.53_{2,42}$	0.22	0.02	$1.53_{1,42}$	0.22	0.01	$2.35_{2,42}$	0.10	0.05	$0.27_{2,42}$	0.76	0.03	$0.61_{1,42}$	0.43	<0.01	$2.40_{2,42}$	0.10	0.05
Shoot: root ratio	$0.90_{2,42}$	0.41	0.01	**$4.49_{1,42}$**	**0.03**	**0.06 (-)**	$0.72_{2,42}$	0.48	0.01	$0.94_{2,42}$	0.39	0.01	$0.84_{1,42}$	0.36	0.01	$1.03_{2,42}$	0.36	0.01
Shoot stoichiometry																		
C: N ratio	**$4.21_{2,41}$**	**0.02**	**0.12 (+)**	**$4.22_{1,41}$**	**0.04**	**0.07 (-)**	**$4.47_{2,41}$**	**0.01**	**0.12 (-)**	$0.36_{2,41}$	0.69	0.02	$0.33_{1,41}$	0.56	0.01	$3.19_{2,41}$	0.05	0.08
C: P ratio	$0.75_{2,41}$	0.47	0.01	**$20.61_{1,41}$**	**<0.01**	**0.29 (-)**	$2.87_{2,41}$	0.06	0.07	$0.01_{2,41}$	0.98	0.04	$1.19_{1,41}$	0.28	<0.01	$2.75_{2,41}$	0.07	0.07
N: P ratio	$1.36_{2,41}$	0.26	0.01	**$12.96_{1,41}$**	**<0.01**	**0.19 (-)**	$0.10_{2,41}$	0.90	0.04	$1.63_{2,41}$	0.20	0.01	$1.63_{1,41}$	0.20	0.01	$0.28_{2,41}$	0.75	0.03
C: N: P ratio	$2.98_{2,41}$	0.06	0.07	**$15.91_{1,41}$**	**<0.01**	**0.24 (-)**	**$4.01_{2,41}$**	**0.02**	**0.11 (-)**	$0.12_{2,41}$	0.88	0.03	$0.65_{1,41}$	0.42	0.01	$3.22_{2,41}$	0.05	0.08
Soil microbial response																		
Bacterial biomass	$0.38_{2,41}$	0.68	0.02	$0.03_{1,41}$	0.85	0.02	$0.03_{1,41}$	0.96	0.04	**$7.50_{2,41}$**	**<0.01**	**0.21 (-)**	$<0.01_{1,41}$	0.95	0.02	$0.03_{1,41}$	0.96	0.04
Fungal biomass	**$4.21_{2,41}$**	**0.02**	**0.11 (-)**	$<0.01_{1,41}$	0.96	0.02	$0.20_{1,41}$	0.81	0.03	**$19.01_{2,41}$**	**<0.01**	**0.43 (-)**	$0.16_{1,41}$	0.68	0.02	$0.46_{1,41}$	0.62	0.02

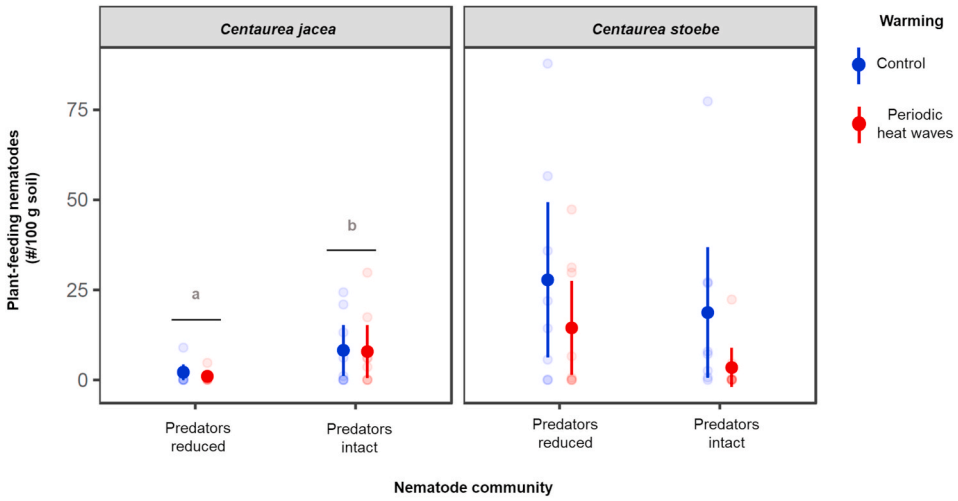

Fig. 1. Density of plant-feeding nematodes of the genus *Coslenchus* (mean ± standard error) in predator reduced and predator intact treatments across two warming scenarios in *Centaurea jacea* (the native plant) and *Centaurea stoebe* (the range-expanding plant). The letters above the bars are based on Tukey post-hoc tests. Nematode densities are based on qPCR estimations (see methods).

native *C. jacea* (Table 1). The difference in shoot C: N ratio of *C. jacea* was most pronounced between the periodic heat waves and ambient temperature treatments in soils where predatory nematodes were reduced (Fig. 2), which correspond to significant interaction between nematode community and temperature treatments (Table 1). While a similar trend was observed also in *C. stoebe*, we did not detect any statistical significance in its shoot C: N ratio (Fig. 2, Table 1). Two-way ANOVA showed only the effect of periodic heat waves on shoot C: P ratio of *C. jacea* (Table 1). However, post-hoc tests showed a similar difference between periodically warmed and ambient temperature in predator reduced treatments as observed in shoot C: N ratio of *C. jacea* (Supplementary figure 5). Moreover, we found that shoot C: N: P ratio of *C. jacea* showed an identical response as that of shoot C: N ratio (Fig. 3, Table 1). The stoichiometric responses of *C. stoebe* were consistently weak in all our treatments (Table 1).

Fungal biomass was lower in both reduced and intact predator communities than in soils without nematodes irrespective of periodic heat waves in both plant species (Table 1, Supplementary figure 6). The soil bacterial biomass also decreased in the presence of nematodes but only so in *C. stoebe* soils (Supplementary figure 7). We found no interaction between periodic heat waves and nematode treatments in driving the biomass of both bacterial and fungal communities (Table 1).

3.3. Correlation between plant-feeding nematodes and shoot C: N ratio

Given that we found densities of plant-feeding *Coslenchus* changing with predator treatments, we tested correlations between changes in plant-feeding nematodes with shoot stoichiometry of *C. jacea.* Among the various measures of shoot stoichiometry, we only found plant-feeding nematode densities to associate with shoot C: N ratio of *C. jacea.* Interestingly, we found negative correlations between the density of *Coslenchus* and shoot C: N ratio of *C. jacea* in predator intact treatments across warming treatments (Fig. 4). This, however, was not true in predator reduced treatments. At control temperature, the shoot

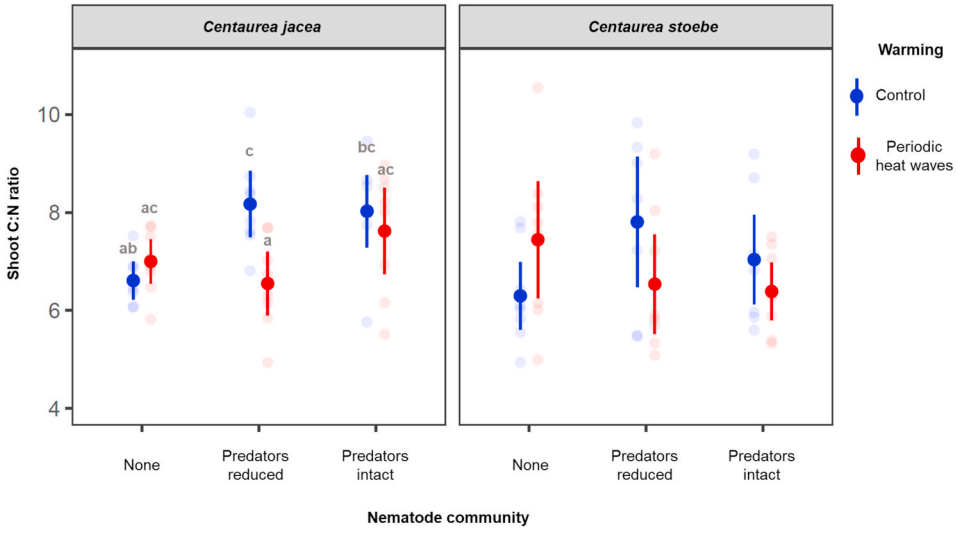

Fig. 2. Effects of nematode community and periodic heat waves on shoot C: N ratio (mean ± standard error) of *Centaurea jacea* (the native plant) and *Centaurea stoebe* (the range-expanding plant). The letters above the bars are based on Tukey post-hoc tests.

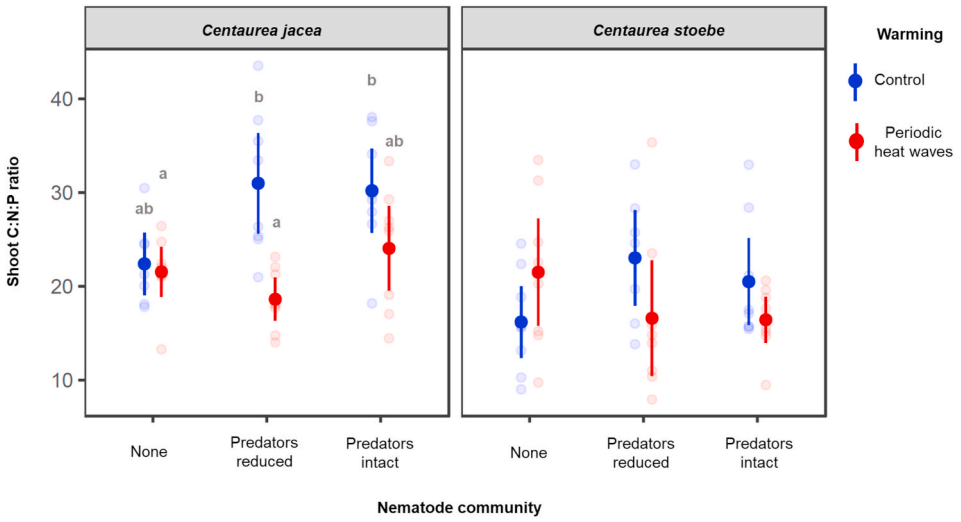

Fig. 3. Effects of nematode community and periodic heat waves on shoot C: N: P ratio (mean ± standard error) of *Centaurea jacea* (the native plant) and *Centaurea stoebe* (the range-expanding plant). The letters above the bars are based on Tukey post-hoc tests.

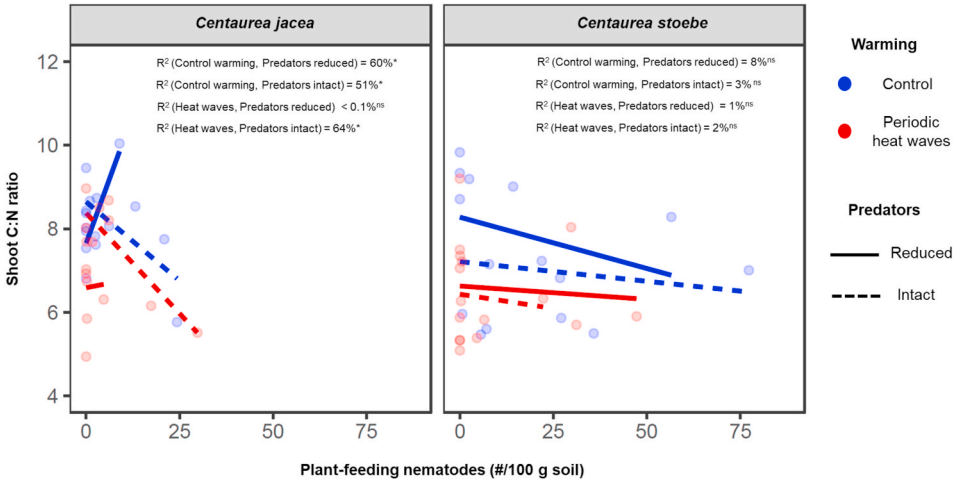

Fig. 4. Correlation between plant-feeding nematodes (*Coslenchus*) and shoot C: N ratio in reduced and intact predator communities for *Centaurea jacea* (the native plant) and *Centaurea stoebe* (the range-expanding plant) in control and periodic heat wave treatments. Nematode densities are based on qPCR estimations (see methods). The shaded area around the correlation lines are standard error. We only found significant correlations in *C. jacea*, which are indicated by asterisk sign on R^2 values. *: p-value<0.05, **: p-value<0.01.

C: N ratio of *C. jacea* appeared to increase with the density of *Coslenchus* nematodes, whereas such a relation disappeared in periodic heat waves (Fig. 4). We did not find any significant correlation between plant-feeding nematodes and shoot C: N ratio in *C. stoebe* (Fig. 4).

4. Discussion

With an increasing frequency and magnitude of climate extremes, ecological communities are constantly exposed to severe abiotic stresses which are likely to change biodiversity and ecosystem functioning (Harris et al., 2018; IPCC et al., 2018). In this study, we show that periodic heat waves alter plant performance by changing their shoot stoichiometry in soils with reduced predatory nematodes. Stoichiometric shifts occurred in a native plant but not in a congeneric range-expanding plant in agreement with our expectation. These shifts corresponded with shifts in plant-feeding nematodes, which were more responsive to predatory treatments than microbial-feeding nematodes. We did not find any effects of nematode communities and periodic heat waves on the biomass of either of the plant species. Our results of stoichiometric shifts suggest that the native plant species *C. jacea* could be more sensitive to environmental changes, such as to the combination of belowground trophic downgrading and climate extremes, than its congener and range-expanding plants *C. stoebe*. We discuss the implications of these results together with underlying processes that may have caused mismatches in stoichiometric responses between the native and the range-expanding plant when exposed to periodic heat waves in soils with reduced predatory nematodes.

Stoichiometric shifts (e.g. increase or decrease in C: N: P ratios) are tightly linked with the fitness of an organism (Sterner and Elser, 2002), and are often an indication of phenotypic plasticity in plant individuals triggered by changes in environments, such as soil nutrient availability (Aerts and Chapin, 2000; Sterner and Elser, 2002). A decline in C: N: P ratios usually mean an increase in the nutritional quality of plants, which are likely to attract plant enemies (Schade et al., 2003) and subsequently influence the fitness of plants (Sardans et al., 2012; Sterner and Elser, 2002). Further, low C: N: P ratios of shoot materials are often more degradable which can have implications for soil carbon dynamics owing to faster decomposition rates (Güsewell and Gessner, 2009; Wardle et al., 2004). Climate warming often enhances soil nutrient turnover in sufficiently wet soils (Bai et al., 2013). A majority of previous studies on climate warming effects on shoot C: N: P ratio have, however, shown a mix of results including a reduction in C: N ratio in moist soils of cold habitats (Sardans et al., 2012). Although only true for the native plant *C. jacea*, a consistent decline in C: N, C: P, N: P and C: N: P ratios at higher temperatures caused by periodic heat waves in our study (Table 1) indicates a clear increase in the nutritional quality of this

native plant. Moreover, in predator reduced soils, the nutritional quality of *C. jacea* shoots were further higher in periodically warmed soils, which disappeared in trophically intact and warmed soils (Figs. 2 and 3). This result implies that soil predator decline and periodic warming together are likely to enhance the forage quality of *C. jacea* for its aboveground herbivores and higher degradability of *C. jacea* litter *via* the decrease in their C: N ratios. Previous studies on *C. stoebe* have indicated that this range-expanding plant contains more toxic compounds to deter belowground herbivory (Wilschut et al., 2016; Wilschut et al., 2017). Therefore, we assumed that weaker effects of predator removal on *C. stoebe* in our study could also relate to stronger bottom-up regulation of belowground herbivory. However, the higher number of herbivore nematodes in *C. stoebe* soils than in *C. jacea* soils refutes this expectation (Fig. 1). We therefore suspect that the range-expanding *C. stoebe* is perhaps lesser responsive to shifts in local soil biotic interactions than its congener native *C. jacea*.

Shifts in stoichiometry of *C. jacea* was correlated with how the predatory nematode reductions influenced the density of dominant plant-feeding nematodes (*Coslenchus*). In general, the density of plant-feeding nematodes was higher in intact predator community in *C. jacea* soils irrespective of warming (Table 1). We suspect two potential reasons for higher numbers of plant-feeding nematodes in predator-intact treatments: first, the initial density of *Coslenchus* was slightly higher in predator intact treatments (Supplementary Table 1) and this difference may have sustained over the experimental period, but mainly so in *C. jacea* soils (Fig. 1). Second, predator presence can also stimulate or maintain higher densities of their prey usually observed for non-specialist predators (Laakso and Setala, 1999). Although for such effects to occur, experimental duration should be long enough to cover several generation times of both predators and prey. While we suspect that our experiment may not have been long enough to capture prey stimulation by predators, predator-induced prey growth stimulation merits further examination in future soil nematode studies.

Moreover, the density of plant-feeding nematodes and shoot stoichiometry of *C. jacea* (namely the C: N ratio) depended on both warming and predator treatments. For instance, the increase in shoot C: N ratio with increase in plant-feeding nematodes was only true when reduced predatory nematodes were in control temperatures (Fig. 4). We suspect that a greater difference in shoot C: N ratio of *C. jacea* between periodically warmed and non-warmed predator reduced treatments could relate to contrasting effects of plant-feeding nematodes on this shoot stoichiometry measurement. Such predator and temperature context-dependent effects of the plant-feeding nematode on shoot stoichiometry implies a complex relationship between root herbivory and plant's strategy of nutrient allocation (McNickle and Evans, 2018). Moreover, the effects of belowground herbivory on plant stoichiometry is still a lesser explored research area when compared to aboveground herbivory effects on plant stoichiometry, which often decreases the plant C: N: P ratio dependent on the herbivory intensity (He et al., 2020). Our results highlight changes in belowground trophic interactions as an important factor for predicting plant's stoichiometric shifts, which we suspect to vary between native and range-expanding plants (Fig. 4).

The biomass responses of both plants in our study in periodically warmed and predator reduced soils were weaker when compared to stoichiometric responses (Table 1). Moderate warming has often been shown to increase plant biomass mainly in colder regions (Dukes et al., 2005; Yue et al., 2017), which are likely triggered by warming-induced greater nutrient turnover in moist and colder soils (Bai et al., 2013). Interestingly, a recent study also pointed out that simulated heat waves only affected the biomass of cold-adapted plants in drier soils (De Boeck et al., 2016). As we constantly supplied water to our experimental pots, it might have reduced the effect size of periodic heat waves on either of the biomass of plants, as usually, heat waves go along with drying of soil.

Microbial biomass decreased in nematode treatments and this decrease was more pronounced in *C. stoebe* soils independent of periodic warming (Table 1). Nematode induced reduction in microbial biomass indicates an expected suppression of soil microbial communities by microbial-feeding nematodes. Over the experimental period, most bacterial-feeding nematodes increased independent of treatments, while fungal-feeding nematodes declined over the experimental period of our study (Supplementary Table 1). Soil bacterial and fungal biomass responses in the presence of their consumers can depend on both direct feeding and indirect effects, such as how the consumers impose competitive interactions between the two groups (Thakur and Geisen, 2019). While our study was not designed to disentangle these effects, lack of predatory nematode effects on microbial biomass responses correspond well with weak effects of predator treatment on microbial-feeding nematodes (Table 1). Greater nematode-induced suppression of bacterial and fungal biomass in *C. stoebe* merits further investigation as it could also relate to unique rhizosphere environments of this range-expanding plant (Wilschut et al., 2016; Wilschut et al., 2017). Our results further suggest that soil warming by periodic heat waves had negligible effect on fungal and bacterial biomass. This is most likely owing to sufficiently maintained soil moisture in our experiments and greater thermal tolerance of soil microorganisms (Bradford, 2013; Thakur, 2020).

The experimental removal of predatory nematodes in our experiment has several limitations, such as variations in the initial density of other groups of nematodes between predator-reduced and predator-intact treatments. Nevertheless, plant-specific responses over the experimental duration point that some effects of predator-reductions were in part driven by altered soil biotic interactions caused by predator loss in warmed soils. Another key limitation is that we were not able to remove juvenile predatory nematodes in predator reduced treatment, which could have compensated for adult predator loss effects over the experimental period. However, large-sized predators (i.e. adult predatory nematodes) are also the more vulnerable ones to disturbance, and our predator-reduced treatment captured this aspect of soil trophic downgrading.

In conclusion, we suggest that loss of adult predatory nematodes could in part shift the shoot stoichiometry of the native plant *C. jacea*, but not of the related range-expander *C. stoebe* when exposed to periodic heat waves. Such stoichiometric shifts may have consequences for plant-herbivore relationships aboveground as well as decomposability of the plant litter material. Previous studies comparing the differences between range-expanding and congeneric native plant species have demonstrated certain general differences between the two plant groups, such as in their ability to evade herbivores (Engelkes et al., 2008), whereas studies have also shown context-dependent differences in chemistry and ecological responses in two plant types to belowground soil organisms (Manrubia, van der Putten, Weser and Veen, 2020; Wilschut et al., 2017). Our results encourage further studies to examine differences between range-expanding and native plant species in more detail in relation to both gradual climate change and climate extremes particularly in disturbed soil habitats. This will help achieve a more complete predictive framework of plant responses to ongoing climate change.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2020.107999.

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